Effects of Digesting Chondroitin Sulfate Proteoglycans on Plasticity in Cat Primary Visual Cortex

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Monocular deprivation (MD) during a critical period of postnatal development produces significant changes in the anatomy and physiology of the visual cortex, and the deprived eye becomes amblyopic. Extracellular matrix molecules have a major role in restricting plasticity such that the ability to recover from MD decreases with age. Chondroitin sulfate proteoglycans (CSPGs) act as barriers to cell migration and axon growth. Previous studies showing that degradation of CSPGs by the bacterial enzyme chondroitinase can restore plasticity in the adult rat visual cortex suggest a potential treatment for amblyopia.

Here MD was imposed in cats from the start of the critical period until 3.5 months of age. The deprived eye was reopened, the functional architecture of the visual cortex was assessed by optical imaging of intrinsic signals, and chondroitinase was injected into one hemisphere. Imaging was repeated 1 and 2 weeks postinjection, and visually evoked potentials (VEPs) and single-cell activity were recorded.

Immunohistochemistry showed that digestion of CSPGs had been successful. After 2 weeks of binocular exposure, some recovery of deprived-eye responses occurred when chondroitinase had been injected into the hemisphere contralateral to that eye; when injected into the ipsilateral hemisphere, no recovery was seen. Deprived-eye VEPs were no larger in the injected hemisphere than in the opposite hemisphere. The small number of neurons dominated by the deprived eye exhibited poor tuning characteristics.

These results suggest that despite structural effects of chondroitinase in adult cat V1, plasticity was not sufficiently restored to enable significant functional recovery of the deprived eye.

Introduction

Amblyopia (“lazy eye”) is the commonest disorder of vision in children. It is usually treated by refractive adaptation, followed by either occlusion (patching) therapy or defocus by atropinization of the good eye, thus disadvantageating it for a time to enforce the use of the amblyopic eye (Stewart et al., 2011). The effectiveness of this procedure decreases with age and is typically limited beyond 8 years. Recently, an increasing number of studies has aimed at treating amblyopia in adulthood by means of restoring visual cortical plasticity (Bavelier et al., 2010).

Adult cortical plasticity is suppressed both at a functional level, through the balance of excitation and inhibition, and at a structural level, through inhibition of dendritic motility and neurite growth (Hensch, 2005). The maturation of the extracellular matrix (ECM) plays a crucial role in terminating cortical plasticity (Silver and Miller, 2004; Laabs et al., 2005). Major components of the ECM in the brain are proteoglycans consisting of a protein core with one or more glycosaminoglycan (GAG) chains attached. Chondroitin sulfate proteoglycans (CSPGs) act as barriers to cell migration (Mizuguchi et al., 2003), and plasticity, mostly through their GAG chains.

During postnatal development, CSPGs accumulate in particular around inhibitory interneurons, contributing to the formation of “perineuronal nets” (PNNs) (Celio et al., 1998). These structures appear to restrict plasticity in the adult brain (Hockfield et al., 1990). In the visual cortex, their appearance coincides with the end of the critical period (Lander et al., 1997), and dark rearing from birth delays not only the critical period but also the expression of antigens associated with PNNs (Guimarães et al., 1990). Mice with attenuated PNNs display persistent ocular dominance plasticity (Carulli et al., 2010). Some of the inhibitory effects of CSPGs appear to be mediated through the Rho/ROCK pathway (Monnier et al., 2003). Furthermore, there are numerous mechanisms by which the GAG chains may interact with growth-inhibitory molecules, as well as cell surface receptors (Properzi and Fawcett, 2004; Sharma et al., 2012).

These findings suggest that a breakdown of CSPGs may reduce inhibition of neurite outgrowth and permit formation or rearrangement of synapses. Indeed, degradation of CSPGs by the bacterial enzyme chondroitinase ABC (ChABC), which cleaves the GAG chains, promotes functional recovery after spinal cord injury (Bradbury et al., 2002). Similarly, degradation of CSPGs by chondroitinase restores experience-dependent plasticity in the adult rat visual cortex, such that monocular deprivation (MD)
imposed after the critical period results in an ocular dominance shift normally observed only in younger animals (Pizzorusso et al., 2002) and rats monocularly deprived during the critical period recover normal ocular dominance if chondroitinase treated in adulthood (Pizzorusso et al., 2006). To translate these results into a potential treatment for humans, we tested this method on cats, a species whose visual cortex has a functional architecture more similar to humans and which develops amblyopia under similar circumstances. We employed optical imaging of intrinsic signals, visual evoked potential, and single-cell recordings to assess functional outcomes.

**Materials and Methods**

All procedures were carried out in accordance with UK Home Office regulations on animal experimentation [Animals (Scientific Procedures) Act 1986] and the European Communities Council Directive 86/609/EEC. Cats of either sex were raised in a normal 12 h light/dark cycle. At postnatal days (P)20 –25 they underwent monocular deprivation by lid suture of the right eye under general anesthesia (ketamine, 20 – 40 mg/kg i.m.; xylazine, 2– 4 mg/kg i.m.). Animals continued to be raised in a normal light/dark cycle until at least P90; they were inspected daily to ensure that the deprived eye remained shut.

**Optical imaging.** All surgical procedures were performed under sterile conditions. Anesthesia was induced with an intramuscular injection of ketamine (20–40 mg/kg) and xylazine (2–4 mg/kg). Atropine (0.2 mg/kg) was injected intramuscularly to reduce mucous secretion. Dexamethasone was injected subcutaneously to prevent cortical edema. Animals were intubated orally and ventilated artificially with a mixture of N₂O:O₂ (60:40) and isoflurane (2.0–3.0%, decreased to 1.2–1.5% during imaging). Rectal temperature (37.5–38.0°C), electrocardiogram (150 –200 beats per minute), and electroencephalogram were monitored throughout the experiment, and adequate measures taken if any of the values diverged from the targets. A 4% glucose in saline solution was infused i.v. at 3 ml/kg/hr throughout the experiment.

Initially, the exposed brain was illuminated with green light, and a reference image of the surface vascular pattern was taken. Subsequently,
the cortex was illuminated with red light at 700 nm. Images were captured using an enhanced differential imaging system (Imager 2001, Opti-
cal Imaging) with the camera focused 500 μm below the cortical surface. Visual stimuli were
produced by a stimulus generator (VSG Three, Cambridge Research Systems), and consisted of
high-contrast (mean luminance, 38 cd/m²), si-
nusoidally modulated gratings of two spatial fre-
quencies [0.1–0.75 cycles/degree (c/deg)] and
four different orientations (0, 45, 90, and 135°)
drifting at 2 Hz. These stimuli were presented
separately to the two eyes using computer-
controlled shutters in randomized sequence and interleaved with trials in which the screen was
blank. Image acquisition lasted for 2 h.

Single-condition responses for each eye were
divided (1) by responses to the blank screen, and (2) by the sum of responses to all four ori-
entations (“cocktail blank”) to obtain iso-
orientation maps. These were then bandpass
filtered and range fitted for display purposes, and orientation angle maps were obtained by
vectorial addition, on a pixel-by-pixel basis, of
the four iso-orientation maps (Bonhoeffer and
Grinvald, 1996).

To quantify cortical territory occupied by
the two eyes, for each hemisphere a region of
interest (ROI) was defined using Interactive
Data Language (IDL) software (Research Sys-
tems), excluding blood vessel and other arti-
facts (Bonhoeffer et al., 1995). Ocular
dominance maps were calculated by dividing
all responses to one eye by the responses to the other. The signal used for
subsequent quantitative analysis was reflectance change (∆R/R) for each
pixel given at 16-bit precision. For analysis of the relative strength of re-
sponses through the two eyes, images were only low-pass filtered
(smoothed). For analysis of areas responding preferentially through one or
the other eye, images were additionally high-pass filtered well above the
periodicity of ocular dominance domains (cutoff, 200 pixels = 7.8 mm) to
level the image intensity across the region of interest. Within the ROI, pixels
were assigned to the left and right eye, respectively, depending on whether
their response ratio was >1 or <1. The numbers of pixels responding more
strongly to the left and the right eye, respectively, were calculated for each
hemisphere. For illustrations, signals were range fitted such that the 1.5%
most responsive (least responsive) pixels were set to black (white), and
Gaussian averaging over six pixels was applied to remove high-frequency
noise. Signal amplitude was displayed on an 8-bit gray scale.

**Chondroitinase treatment.** After completion of the optical imaging ses-
dion, a durotomy was performed (on both cortical hemispheres). Two
or three injections at 1 μL each of ChABC at a concentration of 1000U/ml in
0.9% sterile saline with 0.1% BSA were made into V1 of one hemisphere
as follows. A 5 μL Hamilton syringe with a 33G needle was positioned
terostaxically above the imaged region by means of a 3 axis microma-
nipulator (David Kopf Instruments). A hydraulic microdrive (Narishige)
was adapted to slowly push the plunger down under visual control. In-
jections were spaced about 2–3 mm apart. At each location, 0.5 μL
of ChABC was injected at two different depths, about 500 μm and 1000 μm
below the surface, and the needle was left in place for 5 min before
withdrawal. The imaging chamber was then sealed, the animal treated
with systemic antibiotics (0.1 ml of Metacam, Boehringer-Ingelheim)
analgesics (0.1 ml of Ketofen, Merial); these injections were given
prophylactically for 5 days. Anesthesia was withdrawn, and after a full
recovery the animal was returned to its home cage. After 1 week and again
after 2 weeks the animal was imaged under general anesthesia as de-
scribed above. After the final imaging session, VEP and single-cell re-
cordings were carried out.

**VEP recording.** After imaging data acquisition was completed, the
chamber was reopened and the silicone oil was replaced with saline for

**Figure 2.** Ocular dominance maps in an animal in which the hemisphere contralateral to the deprived eye had been injected with chondroitinase. The top row shows the surface view of the dorsal part of the primary visual cortex on the day when the deprived right eye was reopened and the left hemisphere was injected (stars mark injection sites). It then shows the ocular dominance map for the nondeprived left eye followed by the ocular dominance map for the deprived right eye. Dark regions indicate areas of response through the stimulated eye. Note that the left and right eye maps are negatives of each other; they are both shown to facilitate visual comparison. The middle row illustrates cortical surface and the left and right eye ocular dominance maps 1 week (wk) later; the bottom row shows the same 2 weeks (wks) later. Arrows indicate orientation (A, anterior; R, right). Scale bar, 2 mm.

**Electrophysiology.** In all experimental animals, we determined quanti-
tative orientation/direction tuning curves of single units recorded with
glass-insulated tungsten microelectrodes and discriminated by their
spike shapes using BrainWare software (Tucker-Davis Technologies). Left and right eye responses to drifting sinusoidally modulated gratings
(of optimum spatial frequency) of 16 different directions in 22.5° steps
were averaged over 5 trials of 1.5 s duration. Gaussian tuning curves were
fitted to the data points, and preferred orientation and half-width of
tuning at half-height were determined from these curves. Ocular domi-
nance (OD) categories were assigned to each neuron based on the ratio of
the mean responses to deprived eye (DE) and nondeprived eye (NE)
stimulation, calculated as (DE − NE)/(DE + NE). This yields values
between −1 and 1, which we divided into seven equal intervals to define
OD categories (Hubel and Wiesel, 1962).

External stimulus control, data acquisition, and analysis were per-
formed by TDT System II using BrainWare software (Tucker-Davis
Technologies). Neuronal activity was recorded extracellularly with glass-
insulated tungsten microelectrodes (Ainsworth), which were advanced
into V1 perpendicularly to the cortical surface by means of a stepper motor microdrive (EPS electrode positioning system; Alpha Omega Engineering). This allowed four electrodes to be inserted simultaneously but advanced independently of each other. Recordings were made from neurons throughout the depth of V1. Precise locations of each penetration site were chosen after superimposing the blood vessel patterns on ocular dominance maps obtained in the preceding imaging session. We targeted left and right eye domains on the chondroitinase injected as well as on the control hemisphere. Single units were discriminated by their spike shapes.

**Immunohistochemistry.** After completion of all data acquisition, animals were euthanized and perfused with 4% paraformaldehyde in phosphate buffer and brains were postfixed for two hours before being placed in 30% sucrose in PBS.

In all animals the effectiveness of the treatment was controlled by histology as described (Pizzorusso et al., 2002). Forty micrometer transverse sections from the occipital cortex were cut on a sledge microtome and collected in PBS. Free-floating sections were treated with blocking solution (3% normal horse serum, 0.3% Triton X-100 in 1X PBS) and incubated overnight at 4°C in the monoclonal CSPG stub antibody 1B5 (1:400; Seikagaku, product no. 270431). This was then revealed with 1:500 anti-mouse Alexa Fluor 488 nm (Invitrogen). Alternating sections were treated 1:200 with biotinylated Wisteria floribunda agglutinin (WFA; Sigma L1516) and then with 1:500 streptavidin-Alexa Fluor 568 nm (Invitrogen) or 1:400 monoclonal anti-aggrecan antibody (Millipore Bioscience Research Reagents, catalog no. MAB5284), followed by 1:500 anti-mouse Alexa Fluor 568 nm (Invitrogen). Images were taken with a Leica Leitz DMRB microscope with a Nikon DMX1200 camera.

**Results**

Successful digestion of CSPGs was verified in all ChABC-injected hemispheres by means of immunohistochemistry (Fig. 1). Individual injections resulted in digestion of CSPG side chains, leaving stubs that were then labeled with monoclonal antibody 1B5 in an area of about 1–1.5 mm radius around the injection site and extending throughout all cortical layers. In all animals the extent of digestion covered the area studied by optical imaging. Stub staining revealed CSPG cleavage both in the neuropil and in perineuronal nets surrounding individual neurons. Staining was densest in layer 4, mirroring aggrecan staining of the noninjected hemisphere (see below, this paragraph), but labeled neurons were otherwise randomly distributed within each layer. To quantify ChABC activity, we counted the number of 1B5-positive cells in five fields of view (300 mm x 200 mm) in each of five sections per animal from both the ChABC-injected and the noninjected hemispheres. On the injected side we counted 10.507 ± 0.730 cells (mean ± SD), and on the noninjected side we found 1.253 ± 0.679 cells per field; this difference was highly significant (p < 0.001, homoscedastic *t* test). To assess the effectiveness of digestion we further stained alternating sections for WFA and for aggrecan, the main target of chondroitinase activity. In agreement with previous work (Kind et al., 2012), aggrecan immunoreactivity was strongest in layer 4. Staining was drastically reduced in the chondroitinase-injected hemisphere compared with the noninjected hemisphere. Quantitatively, the number of aggrecan-positive cells was 1.373 ± 0.372 cells per field (mean ± SD) in the injected hemisphere compared with 9.133 ± 0.588 cells in the noninjected hemisphere. This highly significant difference (p < 0.001, homoscedastic *t* test) corresponded to a decrease by 85.0%, demonstrating the effectiveness of chondroitinase action.

**Imaging of cortical maps**

Functional maps of both hemispheres of V1 were obtained by means of intrinsic signal imaging (1) immediately after reopening the deprived eye just before chondroitinase injection, (2) 1 week after the injection, and (3) 2 weeks after the injection. Ocular dominance and orientation preference maps were obtained at all three time points from seven cats in which the hemisphere contralateral to the deprived eye had been injected with ChABC, five cats in which the ipsilateral hemisphere had been injected, and two cats that had not been injected.

Previous studies have shown that, even after just 7–10 days of MD near the peak of the critical period, deprived eye responses become restricted to patches occupying about 20% of the cortical surface and orientation preference maps can no longer be obtained for the deprived eye (Crair et al., 1997; Gillespie et al., 2000; Faulkner et al., 2005; Jaffer et al., 2012).

Ocular dominance maps from one representative cat in which the hemisphere contralateral to the deprived eye had been in-
jected with ChABC are illustrated in Figure 2. As expected, after prolonged monocular deprivation both hemispheres of V1 were strongly dominated by the nondeprived (left) eye and virtually no activity could be elicited through the deprived (right) eye. One week later, some deprived eye responses could be observed in the contralateral (left) hemisphere, and these remained essentially unchanged after a further week. In contrast, in the noninjected ipsilateral hemisphere only minimal responses were seen at both 1 week and 2 weeks after reopening of the deprived eye.

For the seven animals in which the left hemisphere contralateral to the deprived eye had been injected with ChABC, the mean deprived eye territory increased from 13.2 ± 2.0% SEM on the day of the chondroitinase injection to 37.3 ± 6.9% after 1 week and 40.5 ± 8.6% after 2 weeks (Fig. 3A). These increases were significant (both \( p < 0.05 \), paired \( t \) test). For the ipsilateral (noninjected) hemisphere, no significant changes in deprived eye territory were observed (8.6 ± 2.1% after eye opening, 10.2 ± 4.0% after 1 week, and 9.0 ± 2.7% after 2 weeks; both \( p > 0.1 \), paired \( t \) test).

The recovery of deprived eye responses in the contralateral hemisphere could have occurred independently of any chondroitinase action simply through residual cortical plasticity promoted by binocular vision (Mitchell et al., 2001). We therefore analyzed two animals in which neither hemisphere had been treated with chondroitinase and found that responses through the formerly deprived eye changed little over 2 weeks after reopening. In the contralateral hemisphere, responsive areas averaged 20.3% after reopening of the deprived eye, 16.8% 1 week later, and 23.7% 2 weeks later, while for the ipsilateral hemisphere the corresponding values were 10.4, 5.6, and 14.4%, respectively (Fig. 3B). In addition, we examined five cats in which the ipsilateral hemisphere was treated with ChABC, one of which is illustrated in Figure 4. Although in this case we observed some areas of deprived eye response in the contralateral (noninjected) hemisphere, these appeared to be qualitatively weaker than those in the animals where that hemisphere had been injected with chondroitinase. Notably, the treated ipsilateral hemisphere exhibited only very weak deprived eye responses.

For the five animals in which the right hemisphere ipsilateral to the deprived eye had been injected with chondroitinase, the mean area dominated by that eye in the injected hemisphere was 15.3 ± 3.7% SEM after eye opening, 9.8 ± 4.0% one week later, and 11.7 ± 3.3% after a further week (Fig. 3C). None of the changes were significant (\( p > 0.05 \), paired \( t \) test). For the hemisphere contralateral to the deprived eye, the area dominated by that eye increased slightly from 14.9 ± 5.1% SEM at the end of the deprivation period to 20.8 ± 6.2% 1 week later and 25.6 ± 8.2% 2 weeks later; however, these increases were not significant (\( p > 0.05 \), paired \( t \) test).

In terms of true functional recovery, the selectivity of neuronal responses through the formerly deprived eye is as important as the territory occupied by that eye. We therefore analyzed iso-orientation maps obtained through either eye and found that although there were small areas of response to deprived eye stimulation both before chondroitinase injection and 1 or 2 weeks later, their location did not vary with grating orientation (Fig. 5A). When iso-orientation maps were divided by the so-called cocktail blank (the sum of responses to all orientations; Bonhoeffer and Grinvald, 1993) to remove nonselective response components from the images, they became flat (Fig. 5B). In other words, there were no orientation selective responses, and this held true for both injected and noninjected hemispheres (Fig. 5). Accordingly, composite orientation preference maps obtained by vectorial addition of individual iso-orientation maps were essentially flat for the deprived eye in all animals and hemispheres, while the nondeprived eye maps exhibited the typical pinwheel layout (Bonhoeffer and Grinvald, 1993).

**VEP recordings**

As a physiological measure of visual acuity (Berkley and Watkins, 1973; Campbell et al., 1973; Harris, 1978), we analyzed VEPs in response to gratings of a range of spatial frequencies in the seven cats in which the hemisphere contralateral to the deprived eye had been treated with chondroitinase. Previous studies have shown that long-term MD virtually eliminates VEPs through the deprived eye across all spatial frequencies (Snyder and Shapley, 1979; Baro et al., 1990). Responses from a representative animal are shown in Figure 6A, and mean responses for all seven animals in Figure 6B. We only occasionally observed weak deprived eye responses to the lowest spatial frequency tested (0.14 c/deg), and these were no stronger and no more consistent for the contralateral hemisphere than for the ipsilateral hemisphere. Similarly, no significant VEP responses were measured at any spatial frequency for either hemisphere in the five cats in which chondroitinase had been injected ipsilaterally to the deprived eye (Fig. 6C).

**Single-cell recordings**

Since optical imaging of both intrinsic signals and VEPs represents the responses of many neurons and therefore the responses of a minority of stimulus-selective deprived eye responses could be swamped, we recorded from individual neurons in both treated and untreated hemispheres of all animals. We targeted electrode pene-
Despite this approach, of a total of 306 cells recorded from injected injection sites, it then shows iso-orientation maps for right (deprived) eye responses to horizontal (0°) gratings and vertical (90°) gratings normalized by subtraction of the response to a gray screen (“blank”). Dark regions indicate areas of response. On the left the second row shows the “polar” orientation preference map obtained by vectorial addition of responses to gratings of 0, 45, 90, and 135°, where the hue indicates each pixel’s preferred orientation, and the brightness indicates its selectivity for orientation. The middle and right images show iso-orientation maps for right (deprived) eye responses to 0 and 90° gratings normalized by subtraction of the response to the cocktail blank (the sum of responses to all orientations through that eye). The third row shows, on the left, the “polar” orientation preference map for the right (nondeprived) eye. The second and third images show iso-orientation maps for left eye responses to 0 and 90° gratings normalized by subtraction of the response to the cocktail blank. B. Maps obtained from the same animal 2 weeks later; all conventions as in A. Arrows indicate orientation (A, anterior; R, right). Scale bar, 2 mm.

Figure 5. Orientation maps in an animal in which the left hemisphere contralateral to the deprived eye had been injected with chondroitinase. A, Maps obtained at the end of the deprivation period. The top row shows the surface view of the dorsal part of the primary visual cortex on the day when the deprived right eye was reopened and the left hemisphere was injected (stars mark injection sites). It then shows iso-orientation maps for right (deprived) eye responses to horizontal (0°) gratings and vertical (90°) gratings normalized by subtraction of the response to a gray screen (“blank”). Dark regions indicate areas of response. On the left the second row shows the “polar” orientation preference map obtained by vectorial addition of responses to gratings of 0, 45, 90, and 135°, where the hue indicates each pixel’s preferred orientation, and the brightness indicates its selectivity for orientation. The middle and right images show iso-orientation maps for right (deprived) eye responses to 0 and 90° gratings normalized by subtraction of the response to the cocktail blank (the sum of responses to all orientations through that eye). The third row shows, on the left, the “polar” orientation preference map for the left (nondeprived) eye. The second and third images show iso-orientation maps for left eye responses to 0 and 90° gratings normalized by subtraction of the response to the cocktail blank (the sum of responses to all orientations through that eye). The third row shows, on the left, the “polar” orientation preference map for the left (nondeprived) eye. The second and third images show iso-orientation maps for left eye responses to 0 and 90° gratings normalized by subtraction of the response to the cocktail blank. B. Maps obtained from the same animal 2 weeks later; all conventions as in A. Arrows indicate orientation (A, anterior; R, right). Scale bar, 2 mm.

Discussion
In long-term monocularly deprived cats, digestion of CSPG GAG side chains by chondroitinase promoted a moderate recovery of visual cortical responses through the deprived eye. In the hemisphere ipsilateral to the deprived eye chondroitinase treatment appeared to have no effect, whereas in the hemisphere contralateral to the deprived eye chondroitinase injection resulted in a greater increase in territory responding to stimulation of that eye than simple reopening of the eye without treatment. However, the responsive areas largely lacked orientation selectivity, and this was reflected in much broader orientation tuning of single-neuron responses through the deprived eye compared with the nondeprived eye. Moreover, the cutoff point

latter had a mean half-width of tuning of 28.0 ± 0.7° SEM, while the former had a mean half-width of 43.0 ± 3.4° SEM). The difference between the two populations was highly significant (p < 0.001, heteroscedastic t test). To ascertain whether the injection per se had an effect on neuronal responses, we also analyzed the responses of 89 neurons from the untreated hemisphere. For 78 cells dominated by the nondeprived eye the orientation tuning half-width was 29.6 ± 1.5° SEM, which was not significantly different from those cells recorded from the treated hemisphere (p > 0.1, homoscedastic t test). However, the tuning of 11 cells (or 12.4% of the total) dominated by the deprived eye was, at 65.4 ± 7.7° SEM, significantly wider than that of the 42 cells recorded from the treated hemisphere (p < 0.01, homoscedastic t test), suggesting that chondroitinase treatment led to some improvement in orientation selectivity.

In addition to orientation tuning, we assessed spatial frequency tuning with gratings of a range of spatial frequencies presented at each cell’s optimal orientation and found that responses of cells dominated by the deprived eye (Fig. 8A) generally peaked at lower frequencies than those dominated by the nondeprived eye (Fig. 8B). The geometric mean of the cutoff frequency of 201 cells recorded from the injected hemisphere and dominated by the nondeprived eye was 2.10 c/deg, and the geometric mean of the cutoff of 37 cells dominated by deprived eye was 1.22 c/deg. For statistical purposes we transformed the cutoff values logarithmically and found that deprived eye and nondeprived eye cutoffs differed significantly (p < 0.001, heteroscedastic t-test). In contrast, there was no difference between cutoffs of the 201 cells dominated by the nondeprived eye and recorded from the treated hemisphere and cutoffs from the 60 neurons recorded from the untreated hemisphere (geometric mean cutoff, 2.15 c/deg).
of spatial frequency tuning of deprived eye-dominated neurons was significantly reduced relative to neurons driven by the non-deprived eye. Visually evoked potentials were largely absent through the deprived eye in both treated and untreated cortical hemispheres. The apparent discrepancy between a moderate recovery of intrinsic optical signals in response to deprived eye stimulation and the lack thereof in the VEPs indicates that input from the formerly deprived eye is being processed in V1 without resulting in much suprathreshold activity that would be reflected in VEPs or a larger number of deprived eye-driven neurons. Only about 50% of the energy expenditure that drives hemoglobin deoxygenation as a major source of the optical signal (Frostig et al., 1990) is due to action potentials (Attwell and Laughlin, 2001).

The limited scope of recovery from long-term MD in cats contrasts with a report on rats where chondroitinase treatment not only restored a normal ocular dominance distribution among single neurons and normal VEPs recorded from V1, but also resulted in normal behavioral visual acuity in the previously deprived eye (Pizzorusso et al., 2006). One difference between that study and ours is the fact that Pizzorusso et al. (2006) sutured the nondeprived eye at the time of the reopening the deprived eye. However we do not believe that this can account for the discrepant outcomes; in fact, in kittens recovery from a brief period of MD is faster when both eyes are open (as was the case in our study) than when reverse lid suture is carried out (Mitchell et al., 2001). More importantly, when binocular vision is restored in cats following reverse occlusion, the initially deprived eye in most cases suffers a loss in acuity while the initially open eye does not recover normal acuity, resulting in bilateral amblyopia (Mitchell et al., 1984; Murphy and Mitchell, 1986). When comparing the extent of recovery from MD between rats and cats it has to be borne in mind that the two species differ significantly in the severity of the effects of MD in the first place. In particular, the reduction in VEP cutoff point and visual acuity (each by about two-thirds of an octave, from 1.1 to 0.7 c/deg; Pizzorusso et al., 2006) is rather modest compared with the dramatic loss of vision observed in kittens monocularly deprived for similar periods who become blind in the deprived eye (acuity < 0.1 c/deg) compared with a normal acuity of around 6–7 c/deg (Mitchell, 1991), equivalent to a reduction by at least six octaves. It appears that the scope for ocular dominance plasticity during the critical period is greater in cats than in rodents, which may not be surprising given the much higher degree of binocularity. On the other hand, residual "adult" plasticity after the end of the classical critical period is much greater in rodents (Sawtell et al., 2003) than in cats, which may explain why there is more capacity for recovery from long-term MD in the former than in the latter. Another important species difference is the presence and absence of OD columns in cats and rodents, respectively. The segregation of left eye and right eye afferents in cats may limit interactions to near column boundaries, and this may either reduce the scope for reorganization or slow it down. Although in our study there were no obvious differences in recovery of deprived eye responses 1 or 2 weeks after chondroitinase treatment, and although it has previously been shown that while chondroitinase remains active for at least 10 days postinjection neurocan is again being glycanated after 7 days (Lin et al., 2008), it cannot be ruled out that after a longer and/or repeated treatment, recovery might have been greater. In addition, it has to be borne in mind that the cellular mechanisms underpinning adult plasticity in rodents are qualitatively different from those operating during the critical period (Frenkel and Bear, 2004; Sato and Stryker, 2008; Ranson et al., 2012) and might

**Figure 6.** VEPs in response to contrast-reversing gratings of different spatial frequencies. **A.** Amplitude of VEPs recorded from one cat in which the right hemisphere ipsilateral to the deprived eye had been injected with chondroitinase. The responses through the left nondeprived eye (LE) exhibit a cutoff at 2.26 c/deg for both the left hemisphere (LH) and the right hemisphere (RH). Responses through the right eye (RE) were not significantly different from those to a blank screen in either hemisphere. **B.** VEP amplitudes averaged across five animals in which the right hemisphere ipsilateral to the deprived eye had been injected with chondroitinase. Mean amplitudes (+ SEM) are plotted against the spatial frequency of the test gratings for both the left, nondeprived eye and the right, deprived eye. **C.** VEP amplitudes averaged across seven animals in which the left hemisphere contralateral to the deprived eye had been injected with chondroitinase; all conventions as in B.
be either not present in adult cats at all or incapable of reversing processes that took place in early development.

While both glutamatergic and GABAergic receptors and their downstream signaling molecules undoubtedly play a crucial role in determining the time course of the critical period, in particular through the excitatory/inhibitory (E/I) balance (Hensch, 2005; Morishita and Hensch, 2008), the fact that their expression levels do not change greatly at the end of the critical period suggests that there are other factors limiting cortical plasticity beyond that point. The extracellular matrix is of particular interest since it changes from a largely soluble network of proteins during early postnatal development to an insoluble one in adolescence (Hockfield et al., 1990). In fact, the CSPG aggrecan was one of the first proteins to be identified whose expression profile is linked to the time course of the critical period. A particular glycosylation state of aggrecan (Matthews et al., 2002) is detected by the monoclonal antibody Cat-301 in perineuronal nets of specific neurons in the lateral geniculate nucleus (LGN) and visual cortex of cats and
monkeys (Hockfield et al., 1983; Hendry et al., 1984), and its level of expression in the LGN is known to be regulated by visual experience (Sur et al., 1988). The onset of aggregan expression in the cat visual cortex also correlates with the decline in experience-dependent plasticity at the end of the critical period, and dark rearing reversibly reduces aggregan expression (Kind et al., 2012). Importantly, long-term MD does not cause decreased aggregan expression (Kind et al., 2012); therefore, the ineffectiveness of chondroitinase treatment cannot be due to an already reduced CSPG expression that might otherwise have prevented recovery from MD. The relatively moderate effect of CSPG digestion in cats on cortical plasticity therefore suggests that other factors need to be tackled that prevent a better recovery from long-term MD.

First, there are additional structural barriers to adult plasticity, primarily myelin-related proteins that restrict axonal growth, such as NgR (McGee et al., 2005), and CSPG related proteins, such as cartilage link protein Crtl1 (Carulli et al., 2010). Their removal has so far only been shown in mice to restore OD plasticity. The only interventions in adult cats that induced an OD shift following MD are cultured astrocyte grafts (Müller and Best, 2012). With respect to amblyopia, a combination of CSPG digestion with visual tasks requiring binocular interaction (Waddingham et al., 2006; Li et al., 2009) may well be similarly effective. In addition, it may be feasible to make the chondroitinase treatment itself more effective by replacing direct injection with either slow release from a fibrin gel (Hyatt et al., 2010) or lentiviral expression (Zhao et al., 2011).

References
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